

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

NILSSON, Brita
AB Stockholms Patentbyrå, Zacco &
Bruhn
P.O. Box 23101
S-104 35 Stockholm
SUÈDE

Date of mailing (day/month/year) 24 November 2000 (24.11.00)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 29772/BN/AT	
International application No. PCT/EP99/03509	International filing date (day/month/year) 21 May 1999 (21.05.99)

1. The following indications appeared on record concerning:

☒ the applicant ☐ the inventor ☐ the agent ☐ the common representative

Name and Address SBL VACCIN AB S-105 21 Stockholm Sweden	State of Nationality SE	State of Residence SE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☒ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address ACTIVE BIOTECH AB Box 724 S-220 07 Lund Sweden	State of Nationality SE	State of Residence SE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned
☐ the International Searching Authority ☒ the elected Offices concerned
☐ the International Preliminary Examining Authority ☐ other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer A. Karkachi
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

To:

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AB Stockholms Patentbyrå, Zacco &
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P.O. Box 23101
S-104 35 Stockholm
SUÈDE

Date of mailing (day/month/year) 24 November 2000 (24.11.00)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 29772/BN/AT	
International application No. PCT/EP99/03509	International filing date (day/month/year) 21 May 1999 (21.05.99)

1. The following indications appeared on record concerning:
☒ the applicant ☐ the inventor ☐ the agent ☐ the common representative

Name and Address
SBL VACCIN AB
S-105 21 Stockholm
Sweden

State of Nationality State of Residence
SE SE

Telephone No.

Facsimile No.

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☐ the International Searching Authority ☒ the elected Offices concerned
☐ the International Preliminary Examining Authority ☐ other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

A. Karkachi

Facsimile No.: (41-22) 740.14.35

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

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NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

NILSSON, Brita
AB Stockholms Patentbyrå, Zacco &
Bruhn
P.O. Box 23101
S-104 35 Stockholm
SUÈDE

Date of mailing (day/month/year) 24 November 2000 (24.11.00)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 29772/BN/AT	
International application No. PCT/EP99/03509	International filing date (day/month/year) 21 May 1999 (21.05.99)

1. The following indications appeared on record concerning:

☒ the applicant

 ☐ the inventor

 ☐ the agent

 ☐ the common representative

Name and Address

SBL VACCIN AB
S-105 21 Stockholm
Sweden

State of Nationality

SE

State of Residence

SE

Telephone No.

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 ☒ the name

 ☒ the address

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Name and Address

ACTIVE BIOTECH AB
Box 724
S-220 07 Lund
Sweden

State of Nationality

SE

State of Residence

SE

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned
<input type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

A. Karkachi

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 01 February 2000 (01.02.00)	
International application No. PCT/EP99/03509	Applicant's or agent's file reference 29772/BN/AT
International filing date (day/month/year) 21 May 1999 (21.05.99)	Priority date (day/month/year) 26 May 1998 (26.05.98)
Applicant CARLIN, Nils et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
15 December 1999 (15.12.99)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer F. Baechler
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 29772/BN/AT	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/EP 99/03509	International filing date (day/month/year) 21/05/1999	(Earliest) Priority Date (day/month/year) 26/05/1998
Applicant SBL VACCIN AB et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.



the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :



contained in the international application in written form.



filed together with the international application in computer readable form.



furnished subsequently to this Authority in written form.



furnished subsequently to this Authority in computer readable form.



the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.



the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2.



Certain claims were found unsearchable (See Box I).

3.



Unity of invention is lacking (see Box II).

4. With regard to the title,



the text is approved as submitted by the applicant.



the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

the text is approved as submitted by the applicant.



the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

as suggested by the applicant.



because the applicant failed to suggest a figure.



because this figure better characterizes the invention.

13

None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 99/03509

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-9, 12, 13 and 16

A method of producing a Thy-A-deficient strain of *Vibrio cholerae* comprising the step of site-directed mutagenesis at the locus of the *ThyA* gene having the nucleotide sequence SeqIdNo:1; A *vibrio cholerae* *thyA*-deficient strain lacking the functionality of the *thyA* gene; said strain comprising one or several episomal autonomously replicating DNA elements having a functional *thyA* gene that enables the strain to grow in the absence of thymine in the growth medium; said strain wherein said element is a plasmid; said strain comprising a foreign *thyA* gene; said strain wherein the foreign *thyA* gene is an *E.coli* gene; said strain wherein said element also comprises a structural gene encoding a homologous or heterologous protein; said strain wherein said protein is selected from LTB and GST26; A nucleotide sequence and encoded protein of a *thyA* gene of *Vibrio cholerae* having essentially the nucleotide sequence SeqIdNo:1; said *Vibrio cholerae* Thy-A protein having the amino acid sequence SeqIdNo:4; a vaccine comprising as immunising component said *thyA*-deficient *Vibrio cholerae* strain.

2. Claims: 10, 14 and 15

A nucleotide sequence of a 5' flanking region of a structural *thyA* of *Vibrio cholerae* having the nucleotide sequence SeqIdNo:2; A protein encoded by said nucleotide sequence; said protein having the amino acid sequence of SeqIdNo:5

3. Claim : 11

A nucleotide sequence of a 3' flanking region of a structural *thyA* of *Vibrio cholerae* having the nucleotide sequence SeqIdNo:3

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/74 C12N15/54 C12N15/31 C12N9/10 C07K14/245
 C07K14/28 C07K14/435 A61K39/106 A61K39/108
 //C12R1/63

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12R C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMPRO2 [Online] E.M.B.L. Databases Accession Number: Y17135, 1 May 1998 (1998-05-01) VALLE E ET AL: "Vibrio cholerae thyA gene" XP002118053	9-13
Y	abstract	1-8
X	EP 0 251 579 A (ENTEROVAX RES PTY LTD) 7 January 1988 (1988-01-07) cited in the application	16
Y	column 6, paragraphs 1,2; claims 17,18	1-8
Y	EP 0 406 003 A (UNIV CORK) 2 January 1991 (1991-01-02) claims 12,13	1-8

	-/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

8 October 1999

Date of mailing of the international search report

05. 11. 99

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

Lonnoy, 0

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ATTRIDGE SR: "Thymine auxotrophy as an attenuating marker in <i>Vibrio cholerae</i> " MICROB PATHOG, vol. 19, no. 1, July 1995 (1995-07), pages 11-18, XP002118052 abstract; table 1 ---	16
P,X	DATABASE EMPRO2 [Online] E.M.B.L. Databases Accession Number: AJ006514, 5 June 1998 (1998-06-05) CARLIN N: "Vibrio cholerae lgt and thyA genes" XP002118055 abstract ---	9-15
P,X	DATABASE EMPRO2 [Online] E.M.B.L. Databases Accession Number: AJ010968, 21 September 1998 (1998-09-21) CARLIN N ET AL: "Vibrio cholerae nptA gene" XP002118054 100% identity in 1187bp overlap with SeqIdNo.3 abstract ---	11
E	WO 99 35271 A (CAMPOS GOMEZ JAVIER ; LEDON PEREZ TALENA YAMILE (CU); RODRIGUEZ GON) 15 July 1999 (1999-07-15) 99.3% identity in 851bp of Seq.Id.No.1 / 99.3% identity in 283aa of Seq.Id.No.4 claims 9,10,18,19; example 2 ---	1,2,9, 12,13,16
A	WO 94 19482 A (GEN HOSPITAL CORP ; HARVARD COLLEGE (US)) 1 September 1994 (1994-09-01) ---	
A	US 5 470 729 A (KAPER JAMES B ET AL) 28 November 1995 (1995-11-28) -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

/EP 99/03509

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 0251579	A	07-01-1988	AU 594161 B	01-03-1990
			DK 318887 A	25-12-1987
			JP 63039588 A	20-02-1988

EP 0406003	A	02-01-1991	JP 3130083 A	03-06-1991

WO 9935271	A	15-07-1999	NONE	

WO 9419482	A	01-09-1994	AU 683454 B	13-11-1997
			AU 6244594 A	14-09-1994
			CA 2156191 A	01-09-1994
			EP 0692031 A	17-01-1996
			JP 8506963 T	30-07-1996
			US 5747028 A	05-05-1998

US 5470729	A	28-11-1995	US 5135862 A	04-08-1992
			US 4935364 A	19-06-1990
			US 5882653 A	16-03-1999
			AT 145005 T	15-11-1996
			AU 656730 B	16-02-1995
			AU 8214791 A	31-12-1991
			CA 2064046 A,C	06-12-1991
			DE 69123027 D	12-12-1996
			DE 69123027 T	13-03-1997
			DK 485591 T	07-04-1997
			EP 0485591 A	20-05-1992
			ES 2095322 T	16-02-1997
			GR 3022127 T	31-03-1997
			JP 5502381 T	28-04-1993
			WO 9118979 A	12-12-1991
			AT 109202 T	15-08-1994
			AU 566528 B	22-10-1987
			AU 2519584 A	06-09-1984
			CA 1324968 A	07-12-1993
			DE 3486326 D	01-09-1994
			DE 3486326 T	17-11-1994
			DK 151284 A	05-09-1984
			EP 0119031 A	19-09-1984
			EP 0581329 A	02-02-1994
			HK 86595 A	09-06-1995
			US 5628994 A	13-05-1997
			US 5399494 A	21-03-1995
			ES 530249 A	01-01-1986
			JP 1913725 C	23-03-1995
			JP 6030569 B	27-04-1994
			JP 60110286 A	15-06-1985
			PT 78190 A,B	01-04-1992
			ZA 8401557 A	31-10-1984

SE 98/00504

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 15/68, C12N 9/10, A61K 39/106

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL, Databas Genbank/DBDJ, accession no. Y17135, Valle E. et al.: 1998-05-01	9,12,13
Y		1-8
A		10,11,14,15
	--	
Y	EP 0406003 A1 (UNIVERSITY COLLEGE, CORK), 2 January 1991 (02.01.91), page 1-2, claims	1-8
	--	
Y	EP 0251579 A2 (ENTEROVAX RESEARCH PTY. LTD.), 7 January 1988 (07.01.88)	1-8
	--	

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international-type search

12 January 1999

Date of mailing of the international-type search report

1999 -01- 15

Name and mailing address of the ISA/
 Swedish Patent Office
 Box 5055, S-102 42 STOCKHOLM
 Facsimile No. +46 8 666 02 86

Authorized officer

Hampus Rystedt
 Telephone No. +46 8 782 25 00

INTERNATIONAL TYPE SEARCH REPORT

Search request No.

SE 98/00504

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5470729 A (JAMES B. KAPER ET AL), 28 November 1995 (28.11.95), column 2, rows 10-60, claims	16
A	--	1-8
A	US 5198343 A (ERIC DEGRYSE), 30 March 1993 (30.03.93), see especially column 2, rows 55-68	1-8
A	GB 2177097 A (GENENCOR INC.), 14 January 1987 (14.01.87), see especially figure 3	1-8

INTERNATIONAL TYPE SEARCH REPORT
Information on patent family members

01/12/98

Search request No.

SE 98/00504

EP	0406003	A1	02/01/91	JP	3130083	A	03/06/91
EP	0251579	A2	07/01/88	AU	594161	B	01/03/90
				DK	318887	A	25/12/87
				JP	63039588	A	20/02/88
US	5470729	A	28/11/95	AT	145005	T	15/11/96
				AU	656730	B	16/02/95
				AU	8214791	A	31/12/91
				CA	2064046	A	06/12/91
				DE	69123027	D,T	13/03/97
				DK	485591	T	07/04/97
				EP	0485591	A,B	20/05/92
				SE	0485591	T3	
				ES	2095322	T	16/02/97
				GR	3022127	T	31/03/97
				JP	5502381	T	28/04/93
				WO	9118979	A	12/12/91
				AT	109202	T	15/08/94
				AU	566528	B	22/10/87
				AU	2519584	A	06/09/84
				CA	1324968	A	07/12/93
				DE	3486326	D,T	17/11/94
				DK	151284	A	05/09/84
				EP	0119031	A,B	19/09/84
				SE	0119031	T3	
				EP	0581329	A	02/02/94
				HK	86595	A	09/06/95
				US	5135862	A	04/08/92
				US	5399494	A	21/03/95
				US	5628994	A	13/05/97
				JP	1913725	C	23/03/95
				JP	6030569	B	27/04/94
				JP	60110286	A	15/06/85
				PT	78190	B	24/04/86
				US	4935364	A	19/06/90
				ZA	8401557	A	31/10/84

INTERNATIONAL TYPE SEARCH REPORT
Information on patent family members

01/12/98

Search request No.

SE 98/00504

US	5198343	A	30/03/93	AT	129285	T	15/11/95
				AU	616637	B	07/11/91
				AU	7658987	A	11/02/88
				DE	3751564	D,T	28/03/96
				DK	408687	A	06/02/88
				EP	0258118	A,B	02/03/88
				SE	0258118	T3	
				ES	2079348	T	16/01/96
				FR	2602792	A,B	19/02/88
				GR	3018296	T	31/03/96
				JP	2772793	B	09/07/98
				JP	10004981	A	13/01/98
				JP	10066575	A	10/03/98
				JP	63233790	A	29/09/88
				FR	2618159	A	20/01/89

GB	2177097	A	14/01/87	NONE
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PATENT COOPERATION TREATY

PCT

RECD 22 SEP 2000

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

PCT

(PCT Article 36 and Rule 70)

15

Applicant's or agent's file reference 29772/BN/AT	FOR FURTHER ACTION		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP99/03509	International filing date (day/month/year) 21/05/1999	Priority date (day/month/year) 26/05/1998	
International Patent Classification (IPC) or national classification and IPC C12N15/74			
Applicant SBL VACCIN AB et al.			

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☒ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 15/12/1999	Date of completion of this report 19.09.2000
Name and mailing address of the international preliminary examining authority: <div style="display: flex; align-items: center;"> <div> European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465 </div> </div>	Authorized officer Bassias, I Telephone No. +49 89 2399 8106



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP99/03509

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-16 as originally filed

Claims, No.:

1-16 as originally filed

Drawings, No.:

1/17-17/17 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

see separate sheet

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
- ☐ copy of the earlier application whose priority has been claimed.
- ☐ translation of the earlier application whose priority has been claimed.
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/03509

been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

see separate sheet

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.
- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- ☐ complied with.
- ☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- ☐ all parts.
- ☒ the parts relating to claims Nos. 1-9,12,13,16.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP99/03509

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9,12,13,16
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-9,12,13,16
Industrial applicability (IA)	Yes:	Claims	1-9,12,13,16
	No:	Claims	-

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item I (Basis of the opinion)

1. The sequence listing (pages 1-4) was also taken into account for examination.

Re Item II (priority)

1. It appears that the priority is validly claimed. Consequently, the following documents are not considered prior art (the document numbering corresponds to their order of citation in the International Search Report):

D5: DATABASE EMPRO2 [Online] E.M.B.L. Databases Accession
Number: AJ006514, 5 June 1998 (1998-06-05) CARLIN N: 'Vibrio
cholerae lgt and thyA genes' XP002118055

D6: DATABASE EMPRO2 [Online] E.M.B.L. Databases Accession
Number: AJ010968, 21 September 1998 (1998-09-21) CARLIN N ET
AL: 'Vibrio cholerae nptA gene' XP002118054

Re Item IV (unity)

1. The present application does not fulfil the requirements of unity of invention (Rule 13.1 PCT). The following (groups of) potential inventions have been recognized:
 - 1) A method of producing a *thyA*-deficient strain of *Vibrio cholerae*; a *V. cholerae thyA*-deficient strain lacking the functionality of the *thyA* gene, said strain comprising one or several episomal autonomously replicating DNA elements having a functional *thyA* gene; said strain wherein said element is a plasmid; said strain comprising a foreign *thyA* gene; said strain wherein the foreign *thyA* gene is an *E. coli* gene; said strain wherein said episomal autonomously replicating DNA element also comprises a structural gene encoding a homologous or heterologous protein; said strain wherein said protein is LTB and GST 26 kD;
A nucleotide sequence of a *thyA* gene of *V. cholerae* having essentially the nucleotide sequence SEQ ID NO:1; a protein encoded by said nucleotide

sequence of the *thyA* gene of *V. cholerae*; said protein having the amino acid sequence SEQ ID NO:4;

A vaccine comprising as an immunising component said *thyA*-deficient *V. cholerae* strain.

claims: 1-9, 12, 13 and 16

- 2) A nucleotide sequence of a 5'-flanking region of a structural *thyA* gene of *V. cholerae* having essentially the nucleotide sequence SEQ ID NO:2; a protein encoded by said nucleotide sequence; said protein having the amino acid sequence SEQ ID NO:5.

claims: 10, 14 and 15

- 3) A nucleotide sequence of a 3'-flanking region of a structural *thyA* gene of *V. cholerae* having essentially the nucleotide sequence SEQ ID NO:3.

claim: 11

Rule 13.2 PCT stipulates that where a group of inventions is claimed the requirement of unity shall be fulfilled only where there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. "Special" technical features are those features that define a contribution which each of the inventions makes over the prior art.

The three groups of potential inventions are all respectively relating to different nucleotide sequences, namely to the nucleotide sequence of the ***thyA* gene**, to the nucleotide sequence of a **5'-flanking region of the *thyA* gene** and to the sequence of a **3'-flanking region of the *thyA* gene**.

The technical feature shared by these claimed potential inventions is that all those three different nucleotide sequences are located at the same genomic region of *V. cholerae*. However, this cannot be considered as a common inventive concept, since it is expected that nucleotide sequences in question in the genome of an organism are "surrounded" by other nucleotide sequences (which may or may not encode proteins). This is in particular the case in bacteria, which normally possess a "dense" genome.

A common inventive concept could only be acknowledged, if the different nucleotide sequences and their encoded proteins have a functional relation to

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP99/03509

each other, i.e., in the present case are all involved in the biosynthesis of deoxyribosylthymidine triphosphate (dTTP) for incorporation into DNA.

However, this appears not to be the case for the nucleotide sequence (and the thereof encoded protein) upstream of the *thyA* gene (5'-flanking region) and the nucleotide sequence located downstream of the *thyA* gene (3'-flanking region).

Consequently, no "corresponding" special technical feature could be found between the three different nucleotide sequences (and the encoded proteins), and hence three different potential inventions (as defined above) relating to the different nucleotide sequences have been identified.

Since the applicant neither restricted the application nor paid additional examination fees in time, **the present International Preliminary Examination Report is limited to potential invention 1)**, as defined above.

Re Item V (novelty/inventive step)

1. Reference is made to the following documents (the document numbering corresponds to their order of citation in the International Search Report):
 - D1: DATABASE EMPRO2 [Online] E.M.B.L. Databases Accession Number: Y17135, 1 May 1998 (1998-05-01) VALLE E ET AL: 'Vibrio cholerae *thyA* gene' XP002118053
 - D2: EP-A-0 251 579 (ENTEROVAX RES PTY LTD) 7 January 1988 (1988-01-07) cited in the application
 - D9: US-A-5 470 729 (KAPER JAMES B ET AL) 28 November 1995 (1995-11-28)
2. Document D1 discloses the DNA sequence of the *thyA* gene of *Vibrio cholerae*. The sequence of SEQ ID NO:1 shows 99.1% identity in an overlap of 1410 bp with said sequence of D1.
The sequence of SEQ ID NO:1 does not only contain the *thyA* gene (839-1687 bp) but also regions lying upstream and downstream of said gene (1-838 bp = SEQ ID NO:2 and 1688-2909 bp = SEQ ID NO:3). Said flanking regions are lacking in D1 and thus the subject-matter of claim 9 is novel over D1. However, it

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP99/03509

falls within the ordinary skills of a person in the art to clone the upstream and downstream region of a known gene. Consequently, the subject-matter of claim 9 is not inventive (Article 33(3) PCT).

3. Document D1 discloses further the amino sequence of the protein encoded by the *thyA* gene of *Vibrio cholerae*. Said sequence is with the exception of the two first amino acids identical to the sequence shown in SEQ ID NO:4. Hence, the subject-matter of claim 13 lacks, if not novelty (Article 33(2) PCT), at least an inventive step (Article 33(3) PCT).
4. Site-directed mutagenesis in the chromosome of different organisms where specific genes can be deleted and/or interrupted by insertion of other nucleotides is considered a standard technique for skilled persons. Exemplarily, document D9 (US5470729) is mentioned where deletions are inserted into the *V. cholerae* chromosome by *in vivo* recombination between a plasmid carrying the desired deletion, with adjacent flanking sequences, and the *V. cholerae* chromosome (see "abstract").
Since, the nucleotide sequence of SEQ ID NO:1, containing *inter alia thyA*, is not inventive (see paragr. 3 of this Item), a method of producing a *thyA*⁻ strain of *V. cholerae* via site-directed mutagenesis using said non-inventive nucleotide sequence (claim 1) lacks also an inventive activity (Article 33(3) PCT).
5. Since the method for producing a *thyA*⁻ strain of *V. cholerae* is not inventive, a *V. cholerae thyA*⁻ strain, which is a Δ *thyA* strain lacking the functionality of the *thyA* gene (claim 2) is also not in accordance to Article 33(3) PCT.
6. Claims 3-8 do not appear to recite technical features which could justify an inventive step.
- 7.1. Document D2 relates *inter alia* to a method for preparing a bacterial strain including a non-antibiotic marker (see column 5, l.8 - column 6, l.16 and claims 17 and 18). The *thyA* gene cloned in a plasmid is selected as a non-antibiotic marker. Preselected bacterial strains which are *thyA*⁻ are transformed with said plasmids containing the marker. Said transformed strains **including *V. cholerae* may be utilised as live oral vaccines**. Since the *thyA*⁻ strains in D2 could possibly differ

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP99/03509

4. A "homologous or heterologous protein" (claim 7) can only be clear if it is defined to which protein the protein of claim 7 is homologous or heterologous.
5. The claims should not contain references to figures (see e.g., SEQ ID NO:1 of Fig.1).



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